^{14}C-Labeling of a Novel Prostacyclin I_1 Derivative, SM-10902

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SUMMARY

(+)-Methyl [2-[(2*R*,3a*S*,4*R*,5*R*,6a*S*)-octahydro-5-hydroxy-4-[(*E*)-(3*S*,5*S*)-3-hydroxy-5-methyl-1-[3-^{14}C]nonenyl]-2-pentalenyl]ethoxy]-acetate ([nonenyl-3-^{14}C]SM-10902) (1) was labeled with carbon-14 for use in mammalian metabolic studies. The synthesis was achieved in 7 steps from [^{14}C]carbon dioxide: including : 1) Grignard reaction, 2) esterification, 3) condensation with dimethyl methylphosphonate, 4) Wittig-Horner reaction, 5) separation of isomers by HPLC. Stereoselective reduction of the protected ketone with sodium borohydride in the presence of cerium (III) chloride and subsequent desilylation produced 1. The overall yield was 11.1% from Ba[^{14}C]CO_3.

Key Words: carbon-14, prostacyclin, PGI_1 derivative, Grignard reaction, Wittig-Horner reaction

INTRODUCTION

The biological potency of prostacyclin I_2 (PGI_2) coupled with its short half-life has resulted in the synthesis of a large number of analogues in search of stable mimics.[1] (+)-Methyl [2-[(2*R*,3a*S*,4*R*,5*R*,6a*S*)-octahydro-5-hydroxy-4-[(*E*)-(3*S*,5*S*)-3-hydroxy-5-methyl-1-nonenyl]-2-pentalenyl]ethoxy]acetate (SM-10902) (Fig.1) [2] is a novel

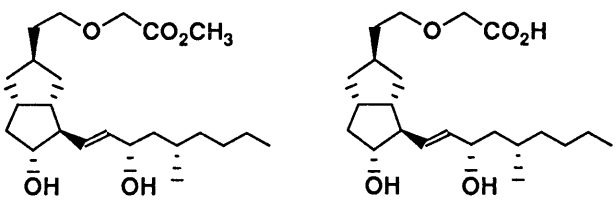

Fig. 1 Structures of SM-10902 and SM-10906

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chemically stable prostacyclin I_1 (PGI_1) derivative whose skin penetrability is expected to be increased by the presence of ester functionality. In rabbit and human serum, SM-10902 is rapidly de-esterified to its free acid, SM-10906 (Fig.1) which inhibits blood platelet aggregation in ADP-induced platelet-rich plasma of human, guinea-pig, dog, rat and rabbit, and this activity is nearly equal to that of prostaglandin E_1 (PGE_1) and less than that of PGI_1.[2b),(3)] For further evaluation of SM-10902, which is a prodrug of SM-10906, as a pharmaceutical, it was required to synthesize radioactive SM-10902 labeled with carbon-14 at the C3 position of the nonenyl group for use in metabolic and pharmacokinetic studies. In this report, we wish to report the synthesis of [nonenyl-3-^{14}C]SM-10902 (1).

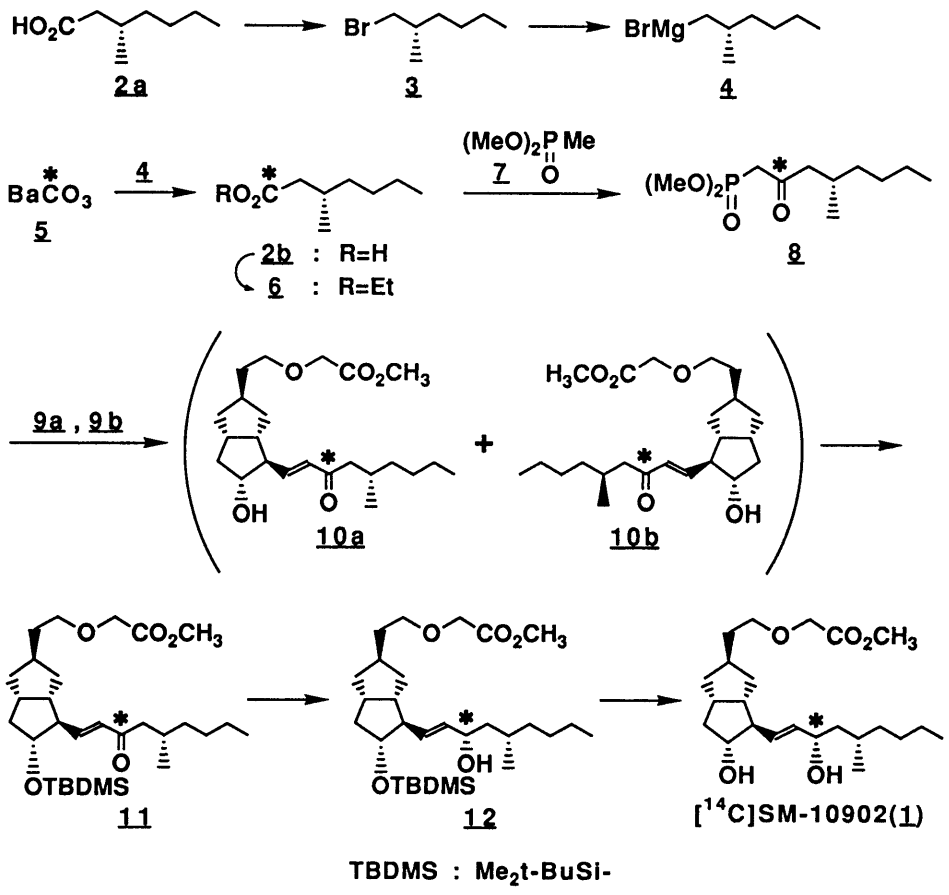

Fig. 2 Synthetic route of [nonenyl-3-^{14}C]SM-10902

RESULTS AND DISCUSSION

The synthetic strategy of radioactive SM-10902 is illustrated in Fig. 2. Several approaches have appeared so far concerning the introduction of ω-side chain of prostacyclins. Among them, Wittig-Horner reaction served as a most promising method because of the easy accessibility of the required phosphonate. Thus, the enone (10a), which was a suitable precursor of 1, was retrosynthetically broken by disconnection of the C1-C2 double bond of the nonenyl group, leading to the aldol (9a) and radioactive ketophosphonate (8). The ester (6) was regarded as a good candidate to afford, in the retrosynthetic direction, the ketophosphonate (8) via condensation with methylphosphonate (7). The ester (6) was traced back to barium carbonate (5), one of the readily accessible radioactive material, and to the optically active Grignard reagent (4).

Our radiosynthesis began with the preparation of the radioactive ketophosphonate (8). The optically active bromide (3) was obtained by Hunsdiecker reaction of the non-radiolabeled acid (2a). Grignard reaction of 4 with [^{14}C]carbon dioxide liberated from barium [^{14}C]carbonate (5) in the usual way gave the acid (2b) in 89% yield.[4] After esterification of 2b, the resulting ester (6) was allowed to react with the anion derived from methylphosphonate (7) to give the ketophosphonate (8) in 86% yield.

On the other hand, the dialdehyde (15), which was a precursor of the unstable non-radiolabeled components (9a and 9b), was prepared as follows (Fig. 3).[2b] The bicyclic ester (13) was converted to the corresponding epoxide (14). Subsequent oxidative ring cleavage of 14 by treatment with periodic acid produced the dialdehyde (15) in 67% yield from 13.

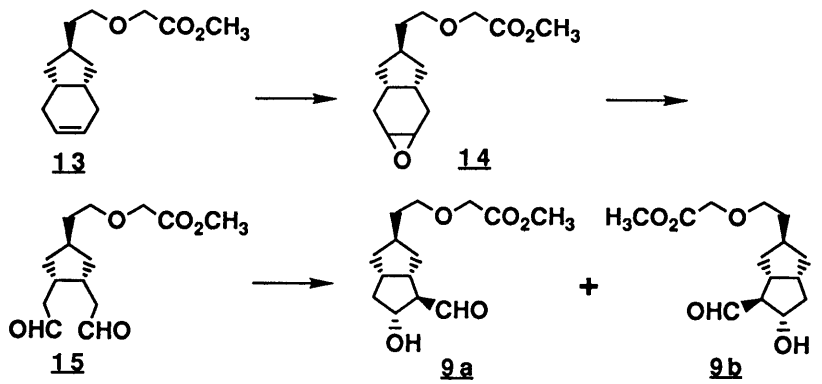

Fig. 3 Synthesis of aldols 9a and 9b

With the two components required for the construction of the carbon skeleton in hand, we proceeded to Wittig-Horner reaction. Intramolecular cyclization of 15 in the presence of piperidine and acetic acid gave the aldols (9a and 9b) as major products. Without separation of the isomers, the mixture was allowed to react with the ketophosphonate (8) to afford enones (10a and 10b) in 57% yield. Construction of the carbon skeleton of 1 was eventually accomplished.

Next we focused on the separation of isomers and the stereoselective reduction of the carbonyl group of the side chain. Reduction of 10a and 10b with L-Selectride showed poor stereoselectivity, giving SM-10902 (1) in 4.9% yield after separation of the isomer. In order to enhance the selectivity of reduction, it was speculated that presence of a bulky protective group for the hydroxyl group would make it possible for a hydride anion to approach only from the side opposite to that protective group. Kawakami et al. already showed that after protection of the hydroxyl group of 10a and 10b with *tert*-butyldimethylsilyl group, reduction of the resulting silyl ethers with sodium borohydride proceeded selectively to yield the desired alcohol. In addition, it was revealed that silyl ethers were effectively separated by using chiral phase HPLC (column CHIRALCEL OD, DAICEL CHEMICAL INDUSTRIES, LTD.).[2b] Thus, after protection of the hydroxyl group in the mixture of 10a and 10b with *tert*-butylchlorodimethylsilane, separation of these compounds by chiral phase HPLC afforded the enone (11) in 45% yield from the mixture of 10a and 10b. Reduction of 11 with sodium borohydride in the presence of cerium (III) chloride[5] below -45°C gave the desired isomer exclusively, which was in sharp contrast to the reduction with L-Selectride. Subsequent desilylation of the resulting alcohol (12) produced [nonenyl-3-^{14}C]SM-10902 (1) in 64% yield from 11. The overall yield was 11.1% from 5, and the specific activity was found to be 2.28 GBq/mmol.

EXPERIMENTAL

Radio-thin layer chromatography (RTLC) was carried out on a Silica Gel F_{254} plate (Merck, Germany), and the radioactivity on the plate was determined by a JTC-601 Radiochromanyzer (Aloka, Japan). Radio-high performance liquid chromatography (RHPLC) was conducted on a LC-3A liquid chromatograph (Shimadzu Co., Ltd., Japan) equipped with a SPD-2A UV detector (Shimadzu Co.) and a RLC-551 Radioanalyzer (Aloka). Radioactivity was measured by a TRI-CARB liquid scintillation counter (Packard Instrument Co., USA) by using Permafluor (Packard) as the counting medium. An infrared spectrum (IR) was measured by a IR-810 grating infrared spectrophotometer (JASCO Co., Ltd., Japan). A proton nuclear magnetic resonance spectrum (NMR) was determined on a Unity 300 spectrometer (Varian, USA) and the

chemical shifts (δ) for protons were quoted in ppm downfield from tetramethylsilane as the internal standard. A mass spectrum (MS) was obtained on a Hitachi M-1000 LC API (Hitachi Ltd., Japan)

(*2S*)-2-Methyl-1-bromohexane (3)

To a solution of (*3S*)-3-methylheptanoic acid (2a) (20.0 g, 139 mmol) in tetrachlorocarbon (100 ml) was added mercury(II) oxide (17.9 g, 82.7 mmol) , and the mixture refluxed for 30 min. To this mixture was added dropwise a solution of bromine (22.2 g, 139 mmol) in tetrachlorocarbon (30.0 ml), and the mixture was stirred at the same temperature for 1 h. After the insoluble materials were filtered off, the filtrate was washed with 5% aqueous sodium hydroxide and saturated sodium chloride solution, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residual oil, which was distilled under reduced pressure (68.5-70.5 °C/30 mmHg) to give 3 (7.34 g, 29.7%).

IR (ν_{max}, cm^{-1}, liquid film): 650 (C-Br)

NMR (δ , ppm, $CDCl_3$): 0.90 (3H, t, J=6.7Hz, $-CH_2CH_3$), 1.01 (3H, d, J=6.6Hz, $-CHCH_3$), 1.17-1.53 (6H, m, $-CH_2CH_2CH_2CH_3$), 1.72-1.88 (1H, m, $-CHCH_3$), 3.27-3.44 (2H, m, $-CH_2Br$)

(*3S*)-3-Methyl[1-^{14}C]heptanoic acid (2b)

Under a nitrogen atmosphere, to a stirred mixture of magnesium turnings (401 mg, 16.5 mmol) and a catalytic amount of iodine in anhydrous tetrahydrofuran (10.0 ml) was added dropwise a solution of the bromide (3) (2.63 g, 14.7 mmol) in anhydrous tetrahydrofuran (20.0 ml) at gentle reflux. After complete addition, the mixture was refluxed for 1 h. The Grignard reagent solution thus obtained was cooled, titrated (0.29 mmol/ml), and charged into two flasks (26 ml and 4 ml, respectively), which were connected to a vacuum manifold and frozen in a liquid nitrogen bath. To one flask containing 7.54 mmol of the Grignard reagent was introduced [^{14}C]carbon dioxide liberated from barium [^{14}C]carbonate (5) (7.47 GBq, 724 mg, 3.67 mmol) with concentrated sulfuric acid. The mixture was warmed to -20°C and stirred at the same temperature for 2 h. The remaining [^{14}C]carbon dioxide gas was then confined in another flask containing 0.6 mmol of the Grignard reagent, and this mixture was also allowed to react under the same condition described above. The reaction mixtures were decomposed with 5% hydrochloric acid, combined and extracted with ether. The organic layer was washed with water and extracted with 5% aqueous sodium carbonate. The alkaline solution was washed with ether, acidified with concentrated hydrochloric acid and extracted with ether. The extract was washed with water and saturated sodium chloride solution and dried over anhydrous sodium sulfate.

Evaporation of the solvent gave 2b (6.64 GBq, 89.2%). The purity was shown to be 99.2% by RTLC (toluene/ethyl acetate/acetic acid=5/7/1 v/v, Rf=0.63).

IR (ν_{max}, cm^{-1}, liquid film): 3300-2500 (COOH), 1710 (CO)

NMR (δ, ppm, $CDCl_3$): 0.89 (3H, t, J=6.3Hz, $-CH_2C\underline{H}_3$), 0.97 (3H, d, J=6.3Hz, $-CHC\underline{H}_3$), 1.30 (6H, m, $-C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 1.96 (1H, m, $-C\underline{H}CH_3$), 2.14 (1H, dd, J=8.4Hz, 15Hz, $-C\underline{H}_2COOH$), 2.36 (1H, dd, J=8.4, 15Hz, $-C\underline{H}_2COOH$), 10.80 (1H, br s, $-COO\underline{H}$)

Ethyl (*3S*)-3-Methyl[1-^{14}C]heptanoate (6)

Under a nitrogen atmosphere, to a stirred solution of the acid (2b) (13.3 GBq, 941 mg, 6.53 mmol) in ethanol (6.5 ml) was added concentrated sulfuric acid (0.15 ml) and the mixture was stirred at 70°C for 4.5 h. After cooling, the mixture was diluted with saturated sodium chloride solution (20 ml) and extracted with ether. The extract was washed with saturated sodium bicarbonate solution, dried over anhydrous sodium sulfate and concentrated at atmospheric pressure to give 6 (12.5 GBq, 94.1%).

IR (ν_{max}, cm^{-1}, liquid film): 1740 (CO)

NMR (δ, ppm, $CDCl_3$): 0.87-0.94 (6H, m, $-CH_2C\underline{H}_3$, $-CHC\underline{H}_3$), 1.17-1.32 (9H, m, $-C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$, $-OCH_2C\underline{H}_3$), 1.95 (1H, m, $-C\underline{H}CH_3$), 2.09 (1H, dd, J=8.1Hz, 15Hz, $-C\underline{H}_2COO-$), 2.29 (1H, dd, J=6.0 Hz, 15Hz, $-C\underline{H}_2COO-$), 4.13 (2H, t, J=7.2 Hz, $-OC\underline{H}_2CH_3$)

Dimethyl (*4S*)-4-Methyl-2-oxo[2-^{14}C]octylphosphonate (8)

Under a nitrogen atmosphere, to n-butyllithium (1.6M in hexane, 16.5 ml, 26.1 mmol) in anhydrous tetrahydrofuran (10.0 ml) was added dropwise a solution of dimethyl methylphosphonate (7) (3.40 g, 27.4 mmol) in anhydrous tetrahydrofuran (5.0 ml) below -60°C, and the mixture was stirred at the same temperature for 1.5 h. To this mixture was added dropwise a solution of the ester (6) (17.7 GBq, 8.72 mmol) in anhydrous tetrahydrofuran (10.0 ml) below -60°C, and the mixture was stirred at the same temperature for 4 h. After dilution with saturated sodium bicarbonate solution, the mixture was warmed to room temperature and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel (hexane/ethyl acetate (2/1 v/v) ~ ethyl acetate) to give 8 (15.2 GBq, 1.87 g, 85.8%). The purity was shown to be 98.2% by RTLC (ethyl acetate, Rf=0.63).

IR (ν_{max}, cm^{-1}, liquid film): 1720 (CO)

NMR (δ, ppm, $CDCl_3$): 0.86-0.91 (6H, m, $-CH_2C\underline{H}_3$, $-CHC\underline{H}_3$), 1.17-1.30 (6H, m, $-C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 2.01 (1H, m, $-C\underline{H}CH_3$), 2.42 (1H, dd, J=8.1Hz, 17Hz,

-CHC$\underline{H_2}$CO-), 2.60 (1H, dd, J=5.7 Hz, 17Hz, -CHC$\underline{H_2}$CO-), 3.07 (2H, dd, J=2.4 Hz, 23Hz, -PC$\underline{H_2}$CO-), 3.80 (6H, d, J=11Hz, -OC$\underline{H_3}$)

Methyl [2-(2*R*, 3a*R*, 7a*S*)-(5,6-epoxy-octahydro-1*H*-inden-2-yl)ethoxy]acetate (14)

To a solution of methyl [2-(2*R*, 3a*R*, 7a*S*)-(2, 3, 3a, 4, 7, 7a-hexahydro-1*H*-inden-2-yl)ethoxy]acetate (13) (10.1 g, 45.0 mmol), prepared according to the method by Kawakami et al.[2b], in dichloroethane (50.0 ml) was added water (8.0 ml), sodium tungstate dihydrate (246 mg, 800 μmol), phosphoric acid (120 μl, 2.0 mmol) and hexadecyltrimethylammonium bromide (144 mg, 400 μmol), and the mixture refluxed for 30 min. To this mixture was added dropwise 20% hydrogen peroxide (13.2 ml, 90.0 mmol) at the same temperature, and the mixture was refluxed for 4 h. After cooling, the mixture was added to a solution of sodium sulfite (5.67 g, 45.0 mmol) in water (30 ml) and the resulting mixture was stirred at room temperature for 15 min. The mixture was extracted with dichloroethane (40 ml), and the organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 14 (9.77 g, 85.7%). The residue thus obtained was subjected to the following reaction without purification.

MS (positive, m/z): 255 $(M+H)^+$.

Methyl {2-(1*R*, 3*R*, 4*S*)-3,4-bis-(2-oxo-ethyl)-cyclopentyl]ethoxy}acetate (15)

To a solution of orthoperiodic acid (12.5 g, 54.8 mmol) in water (30.0 ml) was added the epoxide (14) (9.77 g, 38.4 mmol) in dichloromethane (4.0 ml) at the room temperature, and the mixture was stirred at the same temperature for 4 h. The mixture was extracted with dichloromethane (50.0 ml), and the organic layer was washed with 8% sodium sulfite solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 15 (8.12 g, 78.1%). The residue thus obtained was subjected to the following reaction without purification.

MS (positive, m/z): 271 $(M+H)^+$.

Methyl [2-[(2*R*, 3a*S*, 4*R*, 5*R*, 6a*S*)-octahydro-4-formyl-5-hydroxy-2-pentalenyl]ethoxy]-acetate (9a) and its diastereomer 9b

To a solution of the dialdehyde (15) (9.09 g, 33.6 mmol) in dichloromethane (40.0 ml) was added a solution of piperidine (116 mg, 1.36 mmol) and acetic acid (12 mg, 200 μmol) in dichloromethane (500 μl) at -5~10°C, and the mixture was stirred at the same temperature for 3 h to give the mixture of 9a and its diastereomer 9b. The solution of the mixture of 9a and 9b thus obtained was subjected to the following reaction without purification.

Methyl [2-[(2*R*, 3a*S*, 4*R*, 5*R*, 6a*S*)-octahydro-5-hydroxy-4-[(*E*)-(*S*)-3-oxo-5-methyl-1-[3-^{14}C]nonenyl]-2-pentalenyl]ethoxy]acetate (10a) and its diastereomer 10b

Under a nitrogen atmosphere, to a solution of sodium hydride (60%, 517 mg, 12.9 mmol) in anhydrous tetrahydrofuran (50.0 ml) was added dropwise a solution of the phosphonate (8) (15.2 GBq, 1.87 g, 7.48 mmol) in anhydrous tetrahydrofuran (10.0 ml) at 0°C , and the mixture was stirred at the same temperature for 1 h. To this mixture was added dropwise a solution of the mixture of aldols (9a and 9b) in anhydrous tetrahydrofuran (40.0 ml), and the resulting mixture was stirred at the same temperature for 2 h and further at room temperature for 3 h. After dilution with saturated sodium chloride solution (30.0 ml) , the mixture was extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel (hexane/ethyl acetate (5/1 ~ 1/1 v/v)) to give the mixture of 10a and its diastereomer 10b (8.65 GBq, 1.68 g, 56.8%). The purity of the mixture of 10a and 10b was shown to be 99.4% by RTLC (hexane/ethyl acetate= 1/1 v/v, Rf=0.26).

IR (ν_{max}, cm^{-1}, liquid film): 3450(OH), 1760 (CO)

NMR (δ , ppm, $CDCl_3$): 0.89-0.92 (6H, m, $-CH_2C\underline{H}_3$, $-CHC\underline{H}_3$), 1.14-1.29 (9H, m), 1.58-1.72 (4H, m), 1.99-2.12 (4H, m), 2.23-2.57 (5H, m), 3.49-3.58 (2H, m, $-OC\underline{H}_2CH_2-$), 3.76 (3H, s, $-CO_2C\underline{H}_3$), 3.76-3.86 (H, m, $-C\underline{H}OH$), 4.10 (2H, s, $-OC\underline{H}_2CO_2-$), 6.20 (1H, m, -CH=C<u>H</u>CO-), 6.75 (1H, dd, J=8.4Hz,16Hz, -C<u>H</u>=CHCO-)

Methyl [2-[(2*R*, 3a*S*, 4*R*, 5*R*, 6a*S*)-octahydro-5-(*tert*-butyl-dimethylsiloxy)-4-[(*E*)-(*S*)-3-oxo-5-methyl-1-[3-^{14}C]nonenyl]-2-pentalenyl]ethoxy]acetate (11)

To a solution of the enones (10a and 10b) (8.65 GBq, 1.68 g, 4.25 mmol) in *N*,*N*-dimethylformamide (7.0 ml) was added imidazole(434 mg, 6.37 mmol), *tert*-butyldimethylchlorosilane (769 mg, 5.10 mmol) at room temperature, and the mixture was stirred at the same temperature for 4 h. After dilution with water, the mixture was extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel (hexane ~ hexane/ethyl acetate (1/1 v/v)) to give 11 and its diastereomer. This mixture was separated by HPLC (column CHIRALCEL OD 10 mmID × 25 cm, mobile phase hexane/2-propanol= 1000/8 v/v, flow rate 4.0 ml/min, detector UV (254 nm), retention time 11 : 22 min, diastereomer : 14 min) to give 11 (3.88 GBq, 969 mg, 44.8%). The content of diastereomer was shown to be 99.7% by HPLC (column CHIRALCEL OD 4.6 mmID × 25 cm, mobile phase hexane/2-propanol= 99/1 v/v, flow rate 1.0 ml/min, detector UV (254 nm), retention time 18 min).

NMR (δ , ppm, $CDCl_3$): 0.016 (3H, s, -SiC<u>H</u>$_3$), 0.85-0.91 (15H, m, -CH_2C<u>H</u>$_3$, -CHC<u>H</u>$_3$, -Si$C(CH_3)_3$), 1.12-1.32 (9H, m), 1.55-1.71 (4H, m), 2.03-2.55 (8H, m), 3.53-3.57 (2H, m, -OC<u>H</u>$_2$$CH_2$-), 3.69-3.77 (H, m, -C<u>H</u>OSi-), 3.77 (3H, s, -CO_2C<u>H</u>$_3$), 4.09 (2H, s, -OC<u>H</u>$_2$$CO_2$-), 6.13 (1H, m, -CH=C<u>H</u>CO-), 6.71 (1H, dd, J=8.4Hz,16Hz, -C<u>H</u>=CHCO-)
MS (positive, m/z): 511 $(M+H)^+$.

Methyl [2-[(2*R*, 3a*S*, 4*R*, 5*R*, 6a*S*)-octahydro-5-(*tert*-butyl-dimethylsiloxy)-4-[(*E*)-(3*S*,5*S*)-3-hydroxy-5-methyl-1-[3-^{14}C]nonenyl]-2-pentalenyl]ethoxy]acetate (<u>12</u>)

To a solution of cerium (III) chloride heptahydrate (476 mg, 1.28 mmol) and sodium borohydride (3.6 mg, 0.10 mmol) in methanol (7.0 ml) was added the silyl ether (<u>11</u>) (3.88 GBq, 969 mg, 1.91 mmol) at -45~-50°C , and the mixture was stirred at the same temperature for 30 min. To this mixture was added sodium borohydride (13 mg, 0.33 mmol) every 30 min for four times, and the resulting mixture was stirred at -45~-65°C for 2 h. To the reaction mixture was added dropwise a solution of 1N hydrochloric acid/methanol (20/7 v/v, 2.7 ml) at -30~-50°C and the mixture was stirred at the same temperature for 10 min. After dilution with saturated sodium chloride solution, the mixture was extracted with ethyl acetate. The extract was washed with 5% sodium bicarbonate solution and saturated sodium chloride solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give <u>12</u> (3.87 GBq, 971 mg, 99.7%). The residue thus obtained was subjected to the following reaction without purification.

(+)-Methyl [2-[(2*R*, 3a*S*, 4*R*, 5*R*, 6a*S*)-octahydro-5-hydroxy-4-[(*E*)-(3*S*,5*S*)-3-hydroxy-5-methyl-1-[3-^{14}C]nonenyl]-2-pentalenyl]ethoxy]acetate (<u>1</u>)

Under a nitrogen atmosphere, a solution of the alcohol (<u>12</u>) (3.87 GBq, 971 mg, 1.90 mmol) in methanol/water (1/1 v/v, 6.6 ml) was added acetic acid (11 ml) at room temperature , and the mixture was stirred at the same temperature for 20 h. After dilution with saturated sodium chloride solution (20.0 ml) , the mixture was extracted with ethyl acetate. The extract was washed with saturated sodium bicarbonate solution and saturated sodium chloride solution, dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel (hexane/ethyl acetate (1/1 v/v) ~ ethyl acetate) to give <u>1</u> . The product was further purified by HPLC (column YMC-pack SH-043 S-10 20 mmID × 25 cm, mobile phase hexane/ethanol = 96/4 v/v, flow rate 13.0 ml/min, detector UV (215 nm), retention time 22 min) to give <u>1</u> (2.47 GBq, 656 mg, 64.0%). The radiochemical and chemical purities were shown to be 99.0% and 98.0%, respectively by both methods of RTLC (ethyl acetate, Rf=0.36; acetone/toluene=2/1 v/v, Rf=0.57; chloroform/methanol=20/1 v/v, Rf=0.36) and RHPLC (column SUMIPAX ODS A-211, 5 μ m, 4.6 mmID × 25 cm, mobile phase water/acetonitrile=1/1 v/v, flow rate 1.0 ml/ min, detectors UV (215 nm)

and radiodetector, retention time 34 min; column SUMIPAX YMC GEL SIL 120A, 5 μ m, 6 mmID $\times$ 25 cm, mobile phase hexane/ethanol=96/4 v/v, flow rate 1.0 ml/ min, detectors UV (215 nm) and radiodetector, retention time 20 min).

IR (ν_{max}, cm^{-1}, liquid film): 3375(OH), 1760 (CO)

NMR (δ, ppm, $CDCl_3$): 0.87-0.89 (6H, m, $-CH_2C\underline{H}_3$, $-CHC\underline{H}_3$), 1.12-2.42 (23H, m), 3.54 (2H, t, J=7.0Hz, $-OC\underline{H}_2CH_2-$), 3.60-3.66 (1H, m, $-CH_2C\underline{H}(OH)CH-$), 3.76 (3H, s, $-CO_2C\underline{H}_3$), 4.08 (2H, s, $-OC\underline{H}_2CO_2-$), 4.11 (1H, m, -CH=CH-C<u>H</u>(OH)-), 5.46-5.49 (2H, m, -C<u>H</u>=C<u>H</u>-)

MS (positive, m/z): 399 $(M+H)^+$, 381 $(M-H_2O+H)^+$, 363 $(M-2H_2O+H)^+$.

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